Figure 1: Photograph of the 1.4.4 FTIR spectromicroscopy endstation.

Introduction

The FTIR instrumentation for Beamline 1.4.4 is shown in Figure 1 and consists of a Nicolet Nexus 870 FTIR bench
(to the right), and a
Continuμm XL imaging microscope
(to the left). One of the primary reasons for doing infrared spectroscopy at a synchrotron light source is the large enhancement in brightness (flux per unit area). This brightness advantage is most beneficial when focusing the light to a very small spot size. We have achieved

diffraction-limited spot sizes in the mid-IR using Beamline 1.4.4 (see article).

When you first arrive at the beamline there are several things to do. The main FTIR bench and the IR microscope are always kept powered on, but the IR detector inside the microscope needs to be cooled by filling its dewar with liquid nitrogen. The Continuum XL imaging microscope has two detectors, a single element MCT A* detector and a 28 element focal plane array (FPA) detector. The FPA is not functional with the synchrotron, but can be used with the internal source to obtain fast images with a spatial resolution of ~ 9 µm using the 32x objective. For the best signal to noise and spatial resolution, however, the synchrotron source is required. The rest of this manual will concentrate on how to use the microscope with the synchrotron source. If you are interested in using the array system, please contact Mike or Hans .

To fill the MCT detector,

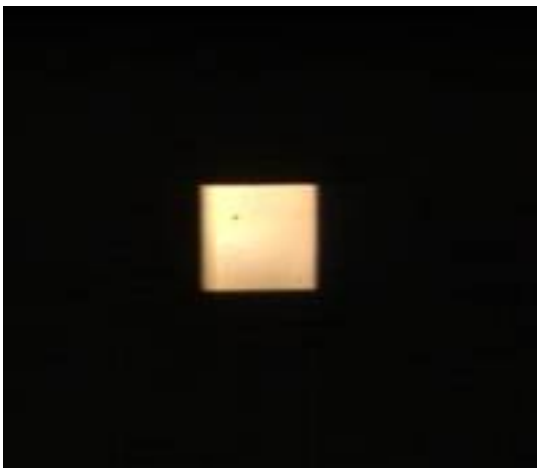
- Put on safety glasses and cryogenic gloves.
- Remove the cap at the position labeled MCT
- Insert the funnel into the dewar (see figure 2).
- Fill the green thermos labeled ‘Nicolet’ with liquid nitrogen using the gray 4 L dewar (the thermos holds approximately the correct amount of liquid nitrogen to fill the MCT detector’s dewar and is easier to handle).
- Pour the liquid nitrogen from the thermos into the funnel. The entire contents of the thermos will not fit in the funnel, so you will have to refill it several times.

If there is no liquid nitrogen in the 4 L dewar, you may fill it at the user liquid nitrogen station (please see the FAQ for directions). The dewar will typically remain cold for > 10 hours, but you should keep track of when you filled it so that the detector does not get warm over a long measurement! It is a good idea to fill the detector during storage ring refills (it will give you something to do).

Figure 2: Cooling the MCT detector with liquid nitrogen.

Reflection Measurements

Select the reflection mode with the black button on the lower front of the microscope. Place a reference sample (gold slide) on the microscope stage, and move the x-y stage using the joystick on its controller to place this sample directly below one of the IR microscope objectives. Turn on the aperture illumination using the knob located furthest from you at the base of the microscope on the left hand side. The aperture image should appear as a square with sharp edges in the center of the screen / eyepiece (see figure 3). If the image is not a square, adjust the focus using the knob located at the base of the microscope on the right hand side. You may have to turn down the intensity of the aperture illumination in order to see the image properly, especially if you are using a gold mirror. You can verify that your sample is properly focused by turning on the reflection illumination knob (middle knob, lower left hand side) and slightly moving the sample using the joystick on the x-y stage controller. If the sample does not move, you have not yet found the correct focus.



Transmission Measurements

The Continuum XL microscope uses matched objectives and condensers for transmission measurements. If you will be using the 15x objective, make sure that the condenser is also 15x. Likewise, the 32x condenser must be used with the 32x objective. Mike or Hans will be happy to show you how to switch between the 15x and 32x condensers.

Assuming the correct condenser is installed, place your sample on the microscope sample stage over a hole (the black three-holed microscope slides will work for this purpose, or any other holder you have that allows light to pass through the sample). If the sample is transparent to the eye then you can use it to align for transmission mode. If not, place your sample over one hole, and make sure there is another hole available on the sample stage for aligning. Adjust the focus such that the sample is directly below one of the upper microscope objectives and focus on the top surface of your sample, as described in the Reflection measurement setup above.

Once the sample is in focus, adjust the condenser focus to collect the transmitted light from the

same location where the upper objective is focusing the incoming light. Move the stage to a hole and select transmission mode with the black button on the lower front of the microscope. Turn on the aperture illumination using the knob located furthest from you at the base of the microscope on the left hand side. Adjust the condenser height (red-labeled knob, base of microscope, right hand side) until aperture image is in focus (it should look like a square with sharp edges). Once the aperture image is in focus, center the aperture on the crosshairs by repositioning the condenser. These adjusters are not very linear and it takes some playing to get used to the way they move the lower aperture. You can tweak the position of the condenser while looking at the IR signal (Collect >> Experimental Setup >> Bench tab) to optimize the signal.

Return the stage to your sample and proceed to the OMNIC section for software control.

Fluorescence Measurements

The Continuμm microscope is equipped with a fluorescence attachment that allows your sample to be observed
under intense fluorescence excitation illumination.

NOTE: The mercury lamp produces UV radiation. Improper use of this device can result in personal injury.

The lamp housing and power supply are normally stored in the cabinet behind the microscope. Please ask Mike or Hans to help you install them properly. Once the housing is installed, it should require minimal adjustment.

To use the fluorescence attachment,

1. Pull out the shutter on the fluorescence attachment to close the light path.
2. Turn on the Power Supply Unit using the large green toggle switch. If the lamp does not turn on, switch the power off once, then turn it back on after 5 -10 seconds. To avoid shortening the lamp lifetime, do not turn the lamp off within 15 minutes of ignition. The lamp typically takes 5-10 minutes to stabilize.
3. Select a cube on the turret that matches the fluorophore of interest. For a list of the excitation/emission properties of the cubes, go here . If you use the WU filter, make sure to use a UV shield and use appropriate eye wear.
4. Turn off the reflection, transmission, and aperture illuminations (lower left hand knobs).
5. Slide the infrared dichroic mirror out (located just below the fluorescence attachment on the right hand side of the microscope). If the fluorescence is intense, you can keep the infrared dichroic mirror in place, but it significantly reduces both the excitation and emission.
6. Select the appropriate objective. You can view fluorescence with the IR objectives and thus directly compare to your infrared image. These objectives, however, are not optimized for fluorescence and as a consequence will make the observed fluorescence weaker.
7. Push in the shutter on the fluorescence attachment to allow light to illuminate your sample.

Note: Do not change filter cubes or objectives when the fluorescence shutter is open!

Note: In order to take IR data, the infrared dichroic mirror must be reinserted (pushed in)!

OMNIC

Please see the OMNIC software manual for a general introduction of the software. There are, however, a few extra things to check on Beamline 1.4.4.

Collect >> Experimental Setup >> Bench Tab

- Detector : Set to MCT A.
- Aperture : Set to Dual. Sometimes the software gets confused and will say that it is in Dual mode even though it is not. If the aperture image does not look rectangular and the sample is in focus, click Single and then return to Dual. This should reset the aperture.

Atlus >> Aperture Size

- Adjust the aperture size. Most users leave the apertures open (~50 µm), but if the system is properly aligned you may close down the aperture to 10 µm to ensure the best spatial resolution. However, this method will result in degraded signal to noise particularly at lower frequencies.